Photopic adaptations to a changing environment in two Lake Victoria cichlids

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During the past 30 years, Lake Victoria cichlid fishes have encountered severe environmental and ecological changes including an introduced predator and new prey types. Furthermore, increased eutrophication has led to reduced water transparency and shifted the spectral composition of underwater light to longer wavelengths. In the present study, collections of two cichlid species, *Haplochromis pyrrhocephalus* and *Haplochromis tanaos*, from before and after the environmental changes, were compared with respect to their photopic resolution and sensitivity. Eyes of both species were dissected and retinal features were measured from tangential sections. In both species, the eyes became smaller, independently of body size. This decrease possibly occurred to make space for other structures that increased in size. In *H. pyrrhocephalus*, a significantly lower resolution was found. However, the size, and thus photon-catching ability of the double cones, remained unchanged, despite the smaller eyes. In the modern populations of *H. tanaos*, the double cone size increased in relation to eye size, so that the photon-capturing ability of the smaller fish remained the same. However, no significant decrease in resolution was found. Shortwave sensitivity was found to be lower in both modern populations because of a reduction or the complete absence of single cones. The results obtained in the present study imply that these resurgent zooplanktivores are capable of adapting their eye morphology to the changed environmental conditions without losing crucial aspects used for survival and reproduction. © 2012 The Linnean Society of London, Biological Journal of the Linnean Society, 2012, 106, 328–341.


INTRODUCTION

Ever since the 1980s, Lake Victoria’s endemic haplochromine cichlids have had to cope with extreme environmental changes in the lake. Nile perch were introduced into the lake and their numbers boomed, resulting in a decline in abundance of the haplochromines (Ogutu-Ohwayo, 1990; Witte et al., 1992; Goudswaard, Witte & Katunzi, 2008). At the same time, eutrophication increased, resulting in lower dissolved oxygen (DO) levels and poorer light conditions (Mugidde, 1993; Hecky et al., 1994; Witte et al., 2005). The increased light absorption by dissolved and dispersed organic matter resulted in reduced illumination and less penetration of short-wavelength blue light (Seehausen et al., 2008). The multiple stressors noted above contributed to the decline of the haplochromines (Kaufman, 1992; Witte et al., 1992; Seehausen, van Alphen & Witte, 1997a; Hecky et al., 2010). However, after a decline of the Nile perch in the 1990s, a number of haplochromine species reappeared in the Mwanza Gulf of Lake Victoria. This occurred despite the fact that predation pressure by the remaining Nile perch was still high, DO concentrations were still low, and the light conditions were still poor (Witte et al., 2000, 2007). Two of the resurgent species were the zooplanktivores *Haplochromis* (*Yssichromis*) *pyrrhocephalus* Witte & Witte-Maas and *Haplochromis tanaos* Van Oijen & Witte. In the 1970s, *H. pyrrhocephalus* co-existed with some other zooplanktivores in the open waters of the Mwanza

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Gulf. It was mainly found near the bottom, at depths of 8–14 m during the day, and nearer to the surface at night (Goldschmidt, Witte & De Visser, 1990). *Haplochromis tanaos* mainly occupied shallow sand bottoms at depths of less than 6 m in bays of the Mwanza Gulf (Van Oijen & Witte, 1996).

After resurgence, *H. pyrrhocephalus* extended its habitat into regions with a depth of only 4 m (M. Kishe-Machumu, pers. comm.; F. Witte and J. C. van Rijssel, pers. observ.) and became one of the more common species (Van Oijen & Witte, 1996; Seehausen et al., 1997b; M. Kishe-Machumu, pers. comm.; F. Witte and J. C. van Rijssel, pers. observ.). In addition, both species shifted their diet from zooplankton to insects and other larger and more robust invertebrates such as shrimps (Van Oijen & Witte, 1996; Katunzi et al., 2003). After resurgence, *H. tanaos* extended its habitat to deeper (13 m) mud bottoms and also became one of the more common species (Van Oijen & Witte, 1996; Seehausen et al., 1997b; F. Witte and J. C. van Rijssel, pers. observ.). In addition, both species shifted their diet from zooplankton to insects and other larger and more robust invertebrates such as shrimps (Van Oijen & Witte, 1996; Katunzi et al., 2003).

Major morphological changes were observed in the resurgent *H. pyrrhocephalus*. An increase in the surface area of the gills appeared to be an adaptation to the lower DO concentrations (Witte et al., 2008). A decrease in head size, which could be an adaptation for escaping Nile perch predation, was also observed (Chapman et al., 2008; Witte et al., 2008; J. C. van Rijssel and F. Witte, unpubl. data). More difficult to explain was a decrease in eye size, despite the decreased light conditions. Witte et al. (2008) suggested that the smaller eye size might be the result of a trade-off with the increased space needed to allocate larger gills and a larger buccal cavity depth in a smaller head. They suggested that the structure of the retina should be studied to determine whether a decrease in eye size would negatively influence the visual capacities of the fish.

There are many ways to compensate for the above mentioned decrease in eye size in a turbid environment. Recent studies have shown that opsin gene expression plays a major role in cichlid eye adaptation and speciation in turbid environments (Carleton et al., 2005; Seehausen et al., 2008; Hofmann et al., 2009, 2010; Maan & Seehausen, 2010). In addition, behavioural changes in response to turbidity can contribute to the persistence of cichlid species (Gray et al., 2011, 2012). However, this is beyond the scope of the present paper, where we concentrate on morphological changes.

To cope with the environmental changes, the recovering haplochromines, which are visual predators (Fryer & Iles, 1972), would require an increased photopic sensitivity. This would mean an enlargement of the cones at the cost of their planimetric and thus angular density (Van der Meer & Anker, 1984). Enlargement of the cones is a common feature in growing cichlid eyes (Van der Meer, 1993, 1994). Thus, the reduced eye size found in the modern population of *H. pyrrhocephalus* was unexpected in relation to the assumed visual demands. Studies of the retina of *H. pyrrhocephalus* from before the environmental changes (old populations) revealed exceptionally large long-wavelength-sensitive (LWS) double cones (Van der Meer, Anker & Barel, 1995). In addition, Van der Meer & Bowmaker (1995) showed that the spectral sensitivity of this species covered significantly longer wavelengths compared to other haplochromines investigated. This suggested a ‘pre-adaptation’ to the new light conditions, which may have contributed to the successful recovery of *H. pyrrhocephalus*.

A decrease in eye size does not necessarily imply a reduction of photopic sensitivity because the photon-catching ability (PhCA) depends on cone-size and not on eye size (Van der Meer & Anker, 1984). An increased PhCA (as a result of increased cone size) compensates for the reduced visibility of nearby objects (predator, prey or congener). The higher the PhCA, the sooner a fish can respond, either by approaching or escaping the object of detection. According to retinal studies on haplochromines reported by Van der Meer et al. (1995), such detection is most profitable in a lateral direction. Therefore, the largest cones were expected to be located in the medial and rostral regions of the retina because the eyes are slightly directed forward. Detail discrimination (e.g. for manipulation of food particles) is determined by resolution, which depends on the angular density of the photopic units (single and double cones; Van der Meer & Anker, 1984). Therefore, the highest angular density of LWS double cones (which are red–green sensitive) was expected in the caudal periphery, creating a detailed image of objects directly in front of the snout.

Blue sensitivity is assumed to have become redundant in the changed spectral environment, and so a reduction in the size and number of single cones was expected because they are known to contain the short-wavelength-sensitive (SWS) photopigments (Van der Meer & Bowmaker, 1995). We also expected to find a square mosaic of double and (small) single cones in the old population of *H. tanaos*. This pattern was also found in *Haplochromis* (*Ptyochromis*) *fischeri* Seegers (formerly *Haplochromis* sauvagei), that used to coexist with *H. tanaos* in the shallow sand habitat. A reduction in the number and size of single cones may provide more room for double cones in a regular cone mosaic (Van der Meer, 1992). Therefore, we expected a modest enlargement of the double cones in the modern population of *H. tanaos* just as in blue-light-deprived specimens of *H. fischeri* (Van der Meer, 1993). To investigate whether the photopic sensitivity
and resolution of the modern populations (collected between 1991 and 2001) of *H. pyrrhocephalus* and *H. tanaos* showed adaptive responses to the new environment, we compared their retinal morphology with those of the old populations (collected between 1977 and 1981).

**MATERIAL AND METHODS**

Adult specimens of both *H. pyrrhocephalus* and *H. tanaos* were collected with bottom trawls in the northern part of the Mwanza Gulf of Lake Victoria over the period 1977–2001. The fish were initially preserved in 10% formalin (buffered with borax) and afterwards kept in 70% ethanol in the Netherlands Centre for Biodiversity, Naturalis, Leiden, for long-term storage. In total, 22 specimens of *H. pyrrhocephalus* and 12 specimens of *H. tanaos* were selected from the period 1977–1981, and 22 specimens of *H. pyrrhocephalus* and 13 specimens of *H. tanaos* were selected from the period 1991–2001. As far as the available material permitted, fish of equal sizes were selected from the different periods. The standard lengths (SL) of these specimens were measured *sensu* Barel et al. (1977). Specimens of *H. pyrrhocephalus* used in the study reported by Witte et al. (2008) were also included in the present study.

The eyes were isolated by dissection and the lens radius (*r*) was measured either by using an eye-piece micrometer mounted on a binocular microscope, or from digital photographs (the two techniques gave identical results). As a result of damage of the retina, not all eyes were suitable for sectioning. Therefore, we selected 20 specimens of old populations (13 *H. pyrrhocephalus* and seven *H. tanaos*) and 22 specimens of modern populations (16 *H. pyrrhocephalus* and six *H. tanaos*) for further analysis (see Supporting information, Tables S1, S2). Whole eyes were dehydrated and embedded in paraplast using the position of the falciform process as a means of orientation. Semi-thin (5 μm) tangential sections were made on a microtome with a steel knife using a graduated location-device (Van der Meer & Anker, 1986) to determine the original position of the sections. The falciform process, which is always located caudo-ventrally, was used as a reference. Accordingly, data were collected from 12 corresponding retinal areas: the medial (*N* = 4) and peripheral (*N* = 8) regions of the dorsal, rostral, ventral, and caudal areas (Fig. 1).

All sections were stained with hematoxylin and eosin to obtain sufficient contrast between inter- and intracellular spaces. The sections were photographed and stored as TIF files. With the use of IMAGE TOOL, version 1.28 (http://ddsdx.uthscsa.edu/dig/itdesc.html) (*H. tanaos*) and IMAGEJ, version 1.44p (http://rsbweb.nih.gov/ij/) (*H. pyrrhocephalus*) the mean size of the double cones (*S_d*; based on five double cones) and their areal density (*D_d*; number per retinal area in three locations; counting was conducted within a field of 10^3 μm^2) were measured and stored. *S_d* was actually the area of the cross-section through the semi-combined ellipsoid of a double cone and was considered to be a measure of photopic sensitivity (Van der Meer & Anker, 1984). The angular density of the double cones (*H_d*; number per degree of visual angle), a measure of retinal resolution, was calculated, using:

\[ H_d = \frac{(2.5 \times r \times \pi \times 360^{-1})^2 \times \pi \times D_d}{(180 \times 360^{-1})^2} \]

(Van der Meer et al., 1995)

The size and angular density of the double cones in the twelve regions were registered for each fish. Measurements on similar retinal locations allowed us to compare individual specimens by mean values. Also the maximum regional values of size (*S_max*) and angular density (*H_max*) were registered for each specimen, as well as their location.

Because not all areal densities of each retina could be measured for *H. tanaos*, missing values were interpolated with the use of known mean densities of the double cones of the other individuals of the same group. By calculating mean relative densities (per retinal position) for other individuals and re-evaluating those according to the other retinal

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**Figure 1.** Schematic schedule of the twelve retinal locations to provide samples for both species (image of *Haplochromis tanaos*). X, centre of retina; C, caudal; D, dorsal; R, rostral; V, ventral; 30, 30°; 60, 60°.
positions, an absolute measure of the unknown areal density was found. Ten missing values for mean density, out of a total of 168 known values, were calculated, assuming an equal retinal distribution amongst individuals.

The size of the inner segments of the single cones ($S_s$) was measured in the regions where they were observed. In these regions, the relation between the number of single and double cones ($s$ $d^{-1}$) was registered. As in a perfect square mosaic $s$ $d^{-1} = 0.5$, this value was referred to as 100% and the measured single cone occupancy was expressed accordingly. The mean value of $S_s$ in each specimen was derived from the observed single cones, if any, in the entire retina. The mean value of $s$ $d^{-1}$ and the percentage of single cone occupancy was derived from the measurements in all twelve regions, including the ones where single cones were absent.

Analysis of variance was used to test whether there was a difference in SL among the selected samples. Because both cone-size and angular density increase during growth, SL and $r$ were chosen as covariates for the general linear model (GLM), with the population period (old or modern) as independent factor, aiming to test for the effect of period on the morphological characters. Dependent variables, independent factors, and the interactions between them were inserted in this selective model. With the use of multivariate analyses of covariance, nonsignificant interactions were removed stepwise from each model and estimated marginal means (EMMs, the means of the morphological characters corrected for the used covariate) were calculated. Significant interactions between SL and population period were plotted to determine the effect of each factor. The unstandardized residuals of each GLM were used to test for normality with the Shapiro–Wilks test. $P$-values of the GLM were corrected with a sequential Bonferroni test. All statistical tests on the morphological characters were performed with SPSS, version 16 (SPSS Inc.).

RESULTS

**HAPLOCHROMIS PYRRHOCEPHALUS**

The mean SL of the modern $H. pyrrhocephalus$ studied (61.4 mm) was slightly but significantly smaller (4.4%) than that of the specimens of the old population (64.2 mm; $P = 0.025$). The lens-radius of the modern population ($r = 1.21$ mm) was also significantly smaller compared to that of the old population ($r = 1.46$ mm; $P < 0.001$) (Table 1). Because of the constant ratio between lens diameter and eye diameter (Matthiessen’s ratio; Otten, 1981), the lens radius serves as a measure for eye size. The relationship between $r$ and SL (Fig. 2, Table 1) showed consistently smaller lenses (17%) in similar sized specimens of the modern population, indicating a significantly smaller eye size compared to the old population.

In seven out of 13 specimens (54%) of the old population, small single cones were sporadically observed and were randomly allocated within the retinal regions (Fig. 3A). In the modern population, except for a few single cones in one specimen, the inner segments or ellipsoids of single cones were never observed (Fig. 3B). In eight out of 16 specimens (50%) of the modern population, relics of single cones (possibly the remains of their nuclei), were sporadically observed in several regions (see Supporting information, Table S1). In the retinas of both the old and modern populations, the double cones were more or less irregularly arranged in rows (Fig. 3A, B). In both populations, the highest densities of double cones were predominantly found in the caudal periphery, whereas the largest cones were mostly measured in the rostral part of the retina (see Supporting information, Table S1).

The $H_d$ and $H_{max}$ (the latter in the caudal periphery) have significantly decreased in the modern population (by 28% and 33%, respectively, $P < 0.001$) (Fig. 2, Table 1). Both the $S_d$ and $S_{max}$ of modern specimens with a small standard length (SL $\leq$ 60 mm) tend to be larger compared to those of the old population (by 15% and 17%, respectively). This occurred while the $S_d$ and $S_{max}$ of larger specimens (SL $> 60$ mm) had decreased in the modern population (by 13% and 19%, respectively), which together results in a significant interaction (Fig. 4). EMMs of $S_d$ and $S_{max}$ differed only slightly between the old and modern populations (Fig. 4, Table 1).

Concerning $H_d$ and $H_{max}$ in relation to $r$, there was no significant effect of period, although there was a significant effect of $r$ as covariate (Table 1). Both $S_d$ and $S_{max}$ showed a significant interaction, with their EMMs differing only slightly (Fig. 5, Table 1).

**HAPLOCHROMIS TANAOS**

The mean SL of the modern $H. tanaos$ (64.7 mm) studied did not differ significantly from that of the specimens of the old population (66.3 mm; $P = 0.289$). The $r$ of the modern population of $H. tanaos$ was 1.10 mm. This value is 9.0% smaller ($P < 0.001$) than that of the old population (1.21 mm) (Fig. 2, Table 2). The single cone size ($S_s$) of the modern population was significantly smaller than in the old population ($P < 0.001$, with SL and $r$ as covariate) (Figs 4, 5). The mean single cone occupancy decreased from 88% to 56% ($P < 0.001$, with SL as covariate), which affected the ratio between single cones and double cones.
### Table 1. Results of the general linear model (GLM) analyses on the morphological characters of *Haplochromis pyrrhocephalus* with standard length (SL) and lens-radius (r) as covariates

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<tr>
<td>Lens-radius (r, mm)</td>
<td>Old</td>
<td>22</td>
<td>1.46</td>
<td>-17.1</td>
<td>0.000</td>
<td>0.000</td>
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<td></td>
<td>Modern</td>
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<td>Angular density (Hd, number per degree)</td>
<td>Old</td>
<td>13</td>
<td>33.6</td>
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<td>31.3</td>
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<tr>
<td></td>
<td>Modern</td>
<td>16</td>
<td>24.1</td>
<td>-28.3</td>
<td>0.000</td>
<td>0.000</td>
<td>NS</td>
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<td>31.8</td>
<td>1.6</td>
<td>NS</td>
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<td>Maximum angular density (Hmax, number per degree)</td>
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<td>13</td>
<td>68.8</td>
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<td>56.8</td>
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<tr>
<td></td>
<td>Modern</td>
<td>16</td>
<td>46.4</td>
<td>-32.6</td>
<td>0.000</td>
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<td>56.5</td>
<td>0.4</td>
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<td>Double cone size (Sd, μm²)</td>
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<td>13</td>
<td>44.3</td>
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<td>41.0</td>
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<td></td>
<td>Modern</td>
<td>16</td>
<td>42.4</td>
<td>-4.3</td>
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<td>13</td>
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<td>7</td>
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<td>Ret. Occ. (%)</td>
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<td>4.0</td>
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<td>Ratio single cones to double cones (sd⁻¹)</td>
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<td>0.04</td>
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<td>Modern</td>
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<td>0</td>
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Significant interactions are shown in italics, significant values after sequential Bonferroni corrections are shown in bold. Means are estimated marginal means derived from the GLM. NS, not significant; Ret. Occ., Retinal occupation of single cones; X, Covariate r not applicable.
(s d<sup>-1</sup>). There was a significant decrease of s d<sup>-1</sup> from 0.44 in the old population to 0.29 in the modern population (P < 0.001) (Table 2).

The loss of single cones did not coincide with a changed configuration from a square pattern into a row pattern (Fig. 3C, D). Square patterns with small or absent single cones were usually transformed into rhombic patterns (intermediate between square and row patterns; Van der Meer, 1992). A clear row pattern was found only on a few occasions in the modern population and only in rostral areas. In both populations, the highest densities of double cones were found predominantly in the dorsal periphery. In the old population, the largest cones were usually observed in the rostral regions of the retina. In the modern population, the largest cones were not strictly confined to a specific region although they were mainly observed in the medial parts (see Supporting information, Table S2).

The H<sub>d</sub> and H<sub>max</sub> (in the dorsal periphery) of the double cones did not show a significant decrease with SL and r as covariates (Figs 2, 5, Table 2). With SL as covariate, S<sub>d</sub> showed a significant interaction, with EMMs of the modern population being slightly larger. No significant difference was found for the S<sub>max</sub> (Fig. 4, Table 2). The S<sub>d</sub> and S<sub>max</sub> were significantly larger in the modern population with r as covariate. However, after Bonferroni correction, the difference for S<sub>max</sub> was no longer significant (Table 2).

**DISCUSSION**

In the resurging populations of *H. pyrrhocephalus* and *H. tanaos*, lens size, and thus eye size, decreased, possibly to permit changes in head morphology for functions other than vision (Witte et al., 2008). Both species showed a lower resolution and a decreased blue SWS light sensitivity. In addition, despite the

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**Figure 2.** Plots of lens-radius (r), mean number of double cones per degree of visual angle (H<sub>d</sub>), and maximum angular density (H<sub>max</sub>) as a function of standard length (SL).
smaller eyes, *H. tanaos* showed an increase of their photopic sensitivity. These changes are in accordance with the increased turbidity, the larger prey types included in the diet of both modern species, and the shift to greater depths in *H. tanaos*.

**CONE SIZE COMPARISON**

There is some discrepancy regarding the double cone size of the old *H. pyrrhocephalus* population from the present study, and that of the sample used by Van der Meer *et al.* (1995). The average double cone size in the old population of *H. pyrrhocephalus* from the latter study was larger (52 µm²) than that in the old population used in the present study (45.5 µm², both not corrected for SL). This difference may have several explanations. Retinal rods and cones exchange places under the influence of the environmental light by contraction or elongation of their myoids (light- and dark-adaptation; Ali, 1975). The specimens used in the earlier study were super-exposed to light when caught. This exposure ensures that they were completely light-adapted (i.e. the cone myoids were maximally contracted, which compressed the ellipsoids towards the outer limiting membrane). This may have been less intense in the light-adapted specimens used in the present study. Moreover, the number of samples from one retina reported by Van der Meer *et al.* (1995) was much larger (over 30 regions) than in the present study (12 regions). This affects the balance between the periphery and the centre of the retina for the benefit of the latter, where also the larger cones are located. Furthermore, the fish specimens (and their eyes) used in the study by Van der Meer *et al.* (1995) were stored in 10% formalin, and were only exposed to alcohol during a relatively short
dehydration step. By contrast, the specimens used in the present study were stored in alcohol for many years, and this may have caused more serious shrinkage of retinal tissue. However, it should be stressed that, in the present study, the techniques used for both old and modern fish were identical, thus making the samples comparable.

**EYE SIZE**

The observed reduction in eye size in the modern population of *H. pyrrhocephalus* (Witte et al., 2008) was confirmed by the present data on lens size in *H. pyrrhocephalus* and *H. tanaos*. The reduction of eye size can be explained by the changed environment. Environmental conditions, especially low dissolved oxygen levels, have a major influence on body shape and cause an increase in gill surface area (Chapman, Galis & Shinn, 2000; Rutjes et al., 2009; Crispo & Chapman, 2010). The first two studies found, under laboratory conditions, larger gills in a larger head. By contrast, Witte et al. (2008) found larger gills in a smaller head in wild modern *H. pyrrhocephalus*. The smaller head may have been caused by the increased predation pressure of Nile perch (Chapman et al., 2008; J.C. van Rijssel and F. Witte, unpubl. data). Moreover, Witte et al. (2008) suggested that the larger buccal cavity depth (cheek depth) could be relevant for eating larger prey items. Consequently, it is likely that the smaller eyes are indirectly caused by predation pressure, diet change, and hypoxia. This means that the smaller eyes act as a trade-off for larger gills and a larger buccal cavity in

![Figure 4. Plots of the mean size of the double cones ($S_d$), maximum regional values of size ($S_{\text{max}}$) and the mean size of the inner segments of the single cones ($S_s$) as a function of standard length (SL).](image-url)
smaller heads (Witte et al., 2008). However, Gray, Sabbah, & Hawryshyn (2011; Gray et al., 2012) found that behavioural changes are also important for survival of cichlids in a turbid environment. Furthermore, female sticklebacks rely more on olfactory than on visual cues in turbid waters when choosing a mate (Heuschele et al., 2009), which might be the case for cichlids too. These studies suggest that there may be multiple strategies to cope with a turbid environment.

**Angular density**

Because visual resolution primarily depends on the number of receptors per visual angle, the lower

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**Figure 5.** Plots of mean number of double cones per degree of visual angle ($H_d$), maximum angular density ($H_{max}$), the mean size of the double cones ($S_d$), maximum regional values of size ($S_{max}$) and the mean size of the inner segments of the single cones ($S_s$) as a function of lens-radius ($r$).
Table 2. Results of the general linear model (GLM) analyses on the morphological characters of *Haplochromis tanaos* with standard length (SL) and lens radius \((r)\) as covariates

<table>
<thead>
<tr>
<th>Character</th>
<th>Population</th>
<th>(N)</th>
<th>Covariate SL</th>
<th>Mean</th>
<th>Difference (%)</th>
<th>(P) Population</th>
<th>(P) SL</th>
<th>(P) Population (\times) SL</th>
<th>Covariate (r)</th>
<th>Mean</th>
<th>Difference (%)</th>
<th>(P) Population</th>
<th>(P) (r)</th>
<th>(P) Population (\times) SL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lens-radius ((r, \text{mm}))</td>
<td>Old</td>
<td>12</td>
<td></td>
<td>1.21</td>
<td>-9.1</td>
<td>0.000</td>
<td>0.000</td>
<td>NS</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Modern</td>
<td>13</td>
<td></td>
<td>1.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Angular density ((H_a, \text{number per degree}))</td>
<td>Old</td>
<td>8</td>
<td></td>
<td>59.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>57.6</td>
<td>2.6</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Modern</td>
<td>6</td>
<td></td>
<td>56.0</td>
<td>-6.5</td>
<td>NS</td>
<td>0.045</td>
<td>NS</td>
<td></td>
<td>59.1</td>
<td>2.6</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum angular density ((H_{max}, \text{number per degree}))</td>
<td>Old</td>
<td>8</td>
<td></td>
<td>98.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>91.8</td>
<td>-2.8</td>
<td>NS</td>
<td>0.072</td>
<td>NS</td>
</tr>
<tr>
<td>Double cone size ((S_d, \text{\mu m}^2))</td>
<td>Modern</td>
<td>6</td>
<td></td>
<td>27.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum double cone size ((S_{max}, \text{\mu m}^2))</td>
<td>Old</td>
<td>8</td>
<td></td>
<td>29.5</td>
<td>7.7</td>
<td>0.029</td>
<td>0.034</td>
<td>0.035</td>
<td></td>
<td>31.7</td>
<td>21.0</td>
<td>0.001</td>
<td>0.000</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Modern</td>
<td>6</td>
<td></td>
<td>39.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>37.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single cone size ((S_s, \text{\mu m}^2))</td>
<td>Old</td>
<td>8</td>
<td></td>
<td>43.3</td>
<td>10.2</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>45.3</td>
<td>19.8</td>
<td>0.019</td>
<td>0.018</td>
<td>NS</td>
</tr>
<tr>
<td>Ret. Occ. (%)</td>
<td>Modern</td>
<td>6</td>
<td></td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio single cones to double cones (\text{\mu d}^{-1})</td>
<td>Old</td>
<td>7</td>
<td></td>
<td>4.2</td>
<td>-40.0</td>
<td>0.000</td>
<td>0.014</td>
<td>NS</td>
<td></td>
<td>4.7</td>
<td>-28.8</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Modern</td>
<td>7</td>
<td></td>
<td>56.0</td>
<td>-36.9</td>
<td>0.000</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>40.8</td>
<td>-53.6</td>
<td>NS</td>
<td>0.050</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Significant interactions are shown in italics, significant values after sequential Bonferroni corrections are shown in bold. Means are estimated marginal means derived from the GLM. Ret. Occ., Retinal occupation of single cones; X, Covariate \(r\) not applicable.
angular density in the modern populations indicates a lower resolution in the modern populations compared to the old population. Because cichlids are known to be visual feeders (Fryer & Iles, 1972), the lower visual resolution is in agreement with the diet shift of *H. pyrrhocephalus* and *H. tanaos* from zooplankton to larger prey types (Van Oijen & Witte, 1996; Katunzi et al., 2003). Differences between old and modern populations in angular density in relation to eye size of both species were caused by similar sized or slightly large double cones in a smaller retina.

**SWS SINGLE CONES**

The reduction of the SWS single cones in both species agrees with the stronger absorbance of shortwave light as a result of eutrophication of the environment (Seehausen, Van Alphen & Witte, 2003) and also with the shift of *H. tanaos* to deeper water over mud bottoms. Apparently, there was no ‘need’ anymore for single cones because the short wavelengths are absorbed by the turbid water. The absence of functional elements of single cones in the studied sections of *H. pyrrhocephalus* does not imply their complete disappearance, as indicated by the occasional nuclei observed in the sections.

A recent study on cone opsin expression in Lake Malawi cichlids revealed that phytozooplanktivores had higher SWS opsin gene expression than species feeding on fish or benthic invertebrates (Hofmann et al., 2009). However, SWS opsin genes of some Lake Victoria cichlid species show hardly any variability between species in contrast to LWS opsin genes (Carleton et al., 2005). These LWS opsin genes were shown to have a high differentiation rate between two sympatric *Pundamilia* phenotypes in association with water clarity (Seehausen et al., 2008). This difference resulted in longer LWS pigments for the deep water red species compared to the shallow water blue species. Multiple studies suggest that the expression of opsin genes might be adaptive and driven by ambient light (Carleton et al., 2005; Seehausen et al., 2008; Hofmann et al., 2009; Maan & Seehausen, 2010). In addition, Hofmann et al. (2010) found evidence suggesting that sensory plasticity played a role in cichlid diversifications in Lake Malawi. Selection on and/or plasticity of opsin genes might also have resulted in the reduction or absence of single cones found in the present study. Unfortunately, our sample did not allow us to study opsin gene expression because fish were preserved in formalin.

Furthermore, it cannot be ruled out that, in the modern populations, the reduction of single cones was the result of the elongation of their myoids (as in dark-adaptation; Ali, 1975). In this scenario, myoid elongation screens the single cones from incoming light and reduces their function. Such a reversible phenotypic phenomenon, however, is not supported by earlier studies of the retina in *H. pyrrhocephalus* raised in brightly illuminated tanks for several generations and showed no single cones (Van der Meer & Bowmaker, 1995).

**LWS DOUBLE CONES**

Even though the eyes became smaller in the modern populations of both species, the double cones remained of a similar size in the adult fish, or even increased for modern *H. tanaos* in relation to the smaller lens-radius. Consequently, the presumed photopic sensitivity did not decrease. The increase in double cone size in *H. tanaos* is likely facilitated by the reduction in size, and decrease in number, of single cones relative to eye size.

The larger size of double cones in the smaller eyes of the modern population of *H. tanaos* suggests a shift in retinal growth from addition to stretching of (cone) cells. *Haplochromis pyrrhocephalus* shows no increase of PhCA (by enlargement of its double cones) as an adaptive adjustment to the darkened environmental light conditions. Thus, the already large cones and LWS photopigments of this species (also observed by Van der Meer & Bowmaker, 1995) might be a ‘pre-adaptation’ to the turbid environment.

The observed maximum double cone size in the medial and rostral regions of the retinas of both species, and the maximum angular density in the caudal periphery of *H. pyrrhocephalus*, are both in accordance with our expectations (see Introduction). They appear to be characteristic for pelagic zooplanktivorous fish (Brownman et al., 1990). The high dorsal resolution in *H. tanaos* (dorsal location of the maximum angular density in both the old and modern populations), suggests a detailed scanning of the bottom. This would categorize this species as a bottom-dweller as also suggested by Van Oijen & Witte (1996) because *H. tanaos* was never caught in surface trawls. The observed retinal findings agree with the relation between retinal cell topography and feeding behaviour in other fish (Shand et al., 2000).

**MECHANISMS BEHIND RETINAL CHANGES**

The retinal changes in the modern populations of both species may have been the result of phenotypic plasticity as observed in shortwave light-deprived specimens of *H. fischeri* in laboratory experiments (Van der Meer, 1993). However, there are several other possibilities.

Based on molecular data, Mzighani et al. (2010) suggested that, in the relatively murky Mwanza Gulf, modern *H. pyrrhocephalus* and *H. laparogramma*
were not reproductively isolated, whereas they were in three other locations with clearer water. If the modern specimens of *H. pyrrhocephalus* from the Mwanza Gulf were hybrids, this may have influenced the size of their double cones, especially because the double cones of *H. laparogramma* with the same SL were smaller (before the environmental changes, adult *H. laparogramma* were larger than adult *H. pyrrhocephalus*; Van der Meer et al., 1995).

Next to phenotypic plasticity and hybridization, natural selection might have played a role in the observed retinal changes, as was suggested for several opsin genes (Carleton et al., 2005; Seehausen et al., 2008; Hofmann et al., 2009; Maan & Seehausen, 2010).

**MORPHOLOGICAL AND ECOLOGICAL CONVERGENCE**

Apart from the reduction of single cones, both species show other examples of morphological convergence. For example, the mean difference in lens size (and thus eye size) between the old populations of both species (17.1%) was almost twice as large as that between the modern populations (9.1%). There was also some convergence for cone size between both species because the difference became slightly smaller for the modern populations (30.1%) compared to the difference between the old populations (38.1%). The morphological convergences agree with the overlap in diet and habitat found for the resurgent populations of both species. Moreover, body shape convergence was found between *H. pyrrhocephalus* and *H. laparogramma* (J. C. van Rijssel and F. Witte, unpubl. data); the latter species shifted its habitat and diet towards that of *H. pyrrhocephalus* (Witte, Goldschmidt & Wanink, 1995; J. H. Wanink, unpubl. data).

**CONCLUSIONS**

*Haplochromis pyrrhocephalus* and *H. tanaos* have shown adaptive ecological responses including a probable reallocation of internal head structures (Witte et al., 2008). In addition, retinal features have changed in such a way that only the resolution and not the sensitivity for the ambient wave lengths) in the modern environment was negatively affected by the changing environment. Whether the morphological and retinal adjustments found in the present study are the result of direct selection pressure, phenotypic plasticity or hybridization remains unclear. Nonetheless, these adaptive responses might have contributed to the recovery of these two zooplanktivores when other species went extinct.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. General data of Haplochromis pyrrhocephalus specimens used in the present study.
Table S2. General data of Haplochromis tanaos specimens used in the present study.

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